ABSTRACT

Name of the Candidate	:	Aijaz Ahmad
Name of the Supervisor	:	Dr. Nikhat Manzoor
Name of the Co-supervisor	:	Prof. Luqman A Khan
Department	:	Biosciences
Title of the thesis	:	Effect of Eugenol and its Derivatives on Growth and Pathogenicity Markers of *Candida albincans*

Fungal infections occur as a result of a complex interaction between the host, pathogen and the environment. Antibiotics have helped in the treatment of infections to a great extent but their indiscriminate use has led to the development of drug resistant pathogens. The emergence of azole resistance in *Candida albicans* and other *Candida* species is a huge crisis today. The antimicrobial activity of plant essential oils and their components is well established against a wide range of microorganisms. Plants and plant products can assist us in confronting the issue of infection and provide a better understanding of mechanisms for the development of novel and effective antimicrobial agents. Present work investigates the antifungal activity and mode of action of eugenol (EUG), and its five derivatives - methyl eugenol (MEUG), eugenyl acetate, myristicin, thymol (THY) and carvacrol (CARV).

EUG and its derivatives were tested for antifungal activity and were found to be effective against both fluconazole (FLC) susceptible and resistant *Candida* isolates. Myristicin and eugenyl acetate were found to be least effective with MICs ranging from 1500-2000 mgL^{-1}. The mean MIC of EUG was 500 mgL^{-1} which reduced down to 350 mgL^{-1}, 100 mgL^{-1} and 50 mgL^{-1} with MEUG, THY and CARV respectively. It was observed that an additional methyl moiety in EUG structure increases its antifungal activity, while the addition of a bulky moiety like acetate or the methylenedioxy group drastically reduces the antifungal activity of EUG. It was also observed that the differences in functional group positions also alter antifungal activity of the compounds. Exposure to EUG and derivatives resulted in the suppression of cell growth with a delayed lag phase and restricted log phase at sub-inhibitory concentrations of the compounds. Cellular growth was completely arrested at the MIC values. Exposure of EUG and derivatives is inhibitory to fungal growth; however the degree of inhibition depends upon the concentration of these compounds and the duration of exposure.

EUG and its derivatives varied in their mechanism of action depending upon the period of exposure. Short exposures of 5-15 minutes resulted in reduced H^+ efflux by the plasma membrane proton pump. An exposure of 1h resulted in membrane leakage while prolonged exposures of 18h resulted in highly

reduced ergosterol content indicating the involvement of ergosterol biosynthesis pathway in mechanism of action of EUG and derivatives. It was also observed that prolonged exposures of 8h, at very low concentrations induce oxidative stress in yeast contributing to further membrane disintegration. From our studies we conclude that EUG and its derivatives induce production of free radicals. The elevation of ROS stimulates the enzyme SOD. An increased SOD activity resulted in an increase in the concentration of H_2O_2 which further stimulates the peroxide eliminating enzyme, primarily GPx. GSH is an essential substrate of GPx. It is noteworthy that the levels of GSH were drastically reduced by the test compounds and this reduction gets even greater as increased levels of H_2O_2 decrease the activity of G6PDH which provides reducing equivalents to GR, an enzyme that recycles GSH from GSSG. As a result, decreased G6PDH activity ultimately aids further in the reduction of GSH. Again, reduced availability of GSH explains decreased GPx activity. The same explanation can be given for decreased activity of GST. Another enzyme characterized to eliminate H_2O_2 is catalase, which triggers a cellular response leading to an increase in its activity. Hence increase in the activity of two important antioxidant enzymes namely SOD and catalase, clearly demonstrates an increase in the concentration of ROS when the *Candida* cell were exposed to the EUG and derivatives. However, these enzymatic responses were not enough to defend the cell completely against such a high rise in ROS and therefore did not meet the required cellular antioxidant demand. Ultimately, the outburst of free radical production led to severe lipid peroxidation. Cell death on exposure to EUG and its derivatives hence may be due to (i) decrease in the rate of H^+ efflux (ii) reduced ergosterol content either due to direct interaction of the test compounds with ergosterol or as a result of an inhibitory effect on the enzymes of ergosterol biosynthesis pathway (iii) Induction of oxidative stress in the cell (iv) These processes impair membrane structure and function and as a result form lesions.

Infection process of *C. albicans* is characterized by crucial pathogenicity markers. The initial process of germ tube induction followed by the secretion of hydrolytic enzymes help in the invasion of the host cells. EUG and its derivatives significantly inhibited these pathogenicity markers even at sub-MIC values. Virulence traits depend upon the cells antioxidant status which was impaired by the test compounds.

Combinational studies were performed to investigate the use of chemo-sensitization by EUG and derivatives to augment the efficacy of conventional antifungal drugs, especially azoles. The test compounds exhibited strong synergism with fluconazole even in fluconazole resistant species. Present study encourages the use of EUG and its derivatives especially THY and CARV for topical application in the management of superficial infections as all the four compounds have negligible cytotoxicity in combination with FLC or KETO in combating other type of *Candida* infections. Further studies using animal models are necessary to see the *in vivo* efficacy of the compounds.